Serum soluble human leukocyte antigen-G in pregnancies complicated by severe preeclampsia

MARZENA LASKOWSKA, JAN OLESZCZUK

Abstract

The aim of this study was to investigate the maternal serum of soluble human leukocyte antigen G (sHLA-G) levels in pregnancies complicated by severe preeclampsia. Patients and methods: The study was conducted on 43 patients with pregnancy complicated by severe preeclampsia (the PRE group). The control group consisted of 30 healthy normotensive patients with singleton uncomplicated pregnancies (the C group). Maternal serum sHLA-G levels were calculated using enzyme-linked immunosorbent assay. Results: There were no statistically significant differences in gravidity, parity, maternal age and height in patient profiles between groups. Maternal weight and BMI were lower in the control group. Systolic and diastolic blood pressures were significantly higher in the study group of preeclamptic pregnant women than in the control group. Maternal serum sHLA-G levels tended to be higher in pregnant women with pregnancies complicated by preeclampsia. The mean values were 28.385 ± 23.257 Units/ml in preeclamptic patients and 21.20 ± 22.410 Units/ml in the healthy controls. Conclusions: Our findings suggest that the increased levels of sHLA-G in maternal serum of preeclamptic women may play a significant role in the aetiology of this pregnancy specific disorder and may suggest the state of excessive inflammation and disturbed the feto-maternal immune balance in pregnancies complicated by severe preeclampsia.

Key words: soluble human leukocyte antigen G (sHLA-G), severe preeclampsia

Preeclampsia affects 7-10% of all pregnancies and is a very serious pregnancy disorder, but the aetiology of this disease is uncertain. Pathophysiological processes underlying preeclampsia are complicated, multifactorial and possibly due to an impairment of trophoblast invasion [1], loss of tolerogenic mechanisms and failed remodelling of the maternal spiral arteries [2].

There is growing evidence that decidual natural killer (NK) cells supply factors necessary for the development and arterial modification of the maternal-fetal interface [3].

It was suggested that HLA-G may be a part of the mechanism that enables trophoblasts to invade without being attacked by the lymphocyte population, decidual NK cells. It is possible that trophoblasts with defective HLA-G expression are prone to attack by decidual NK cells and that it results in shallow trophoblast invasion and abnormal non physiological spiral artery remodelling [4].

Functional role of sHLA-G is still unknown. Its elevated serum levels have been reported during acute rejection episodes following organ transplantation and severe graft-versus-host disease after bone marrow transplantation [5]. It was suggested that both membrane-bound and soluble forms of the human leukocyte antigen protect the fetus from maternal immune attack [6]. HLA-G was presented as a ligand for inhibitory receptors present on uterine natural killer cells (NK), thereby contributing to the maternal-fetal tolerance [7, 8]. HLA-G regulates uterine NK cell cytokine and chemokine production and protects the fetus from uterine NK cell cytolyis [9].

Hackmon et al. [10] found that the decline in HLA-G expression during pregnancy may provoke an autoimmune rejection response which is manifested by changes in the levels of inflammatory substances. Trophoblasts which do not express of HLA-G are susceptible to lysis by the decidual NK cells and are prevented from invading deeply into decidua and spiral arteries.

This reduced expression of HLA-G found in preeclampsia may be a primary cause of shallow trophoblast invasion and failure to convert the spiral arteries [11].

It was also suggested that soluble form of HLA-G might be a factor which anergize alloreactive maternal T cells by binding paternal allopeptides and interacting with T cells [12]. Binding of sHLA class I (HLA-A, B, C and G) molecules to CD8 leads to Fas ligand expression, secretion of its soluble form, and apoptosis of activated immune cells by Fas/Fas ligand interaction and results...
in immunosuppression [13]. Strong suppressive effect of sHLA-G on the alloproliferative response of T cells was reported and it was shown that higher levels of the sHLA-G5 isoform result in better graft acceptance in organ transplantation [14].

Puppo et al. [15] suggest that elevated amounts of sHLA-G molecules in amniotic fluid, which is continuously ingested by the fetus, may be of particular relevance for the induction of tolerance and may play a major role in the immune interplay between mother and fetus.

These interactions also regulate production of cytokine and angiogenic factor by uterine NK, favour implantation and placental vascularization and development processes [16] and have been recently attributed to the HLA-G molecule secreted by trophoblast [17].

The aim of this study was to determine of the soluble human leukocyte antigen G (HLA-G) levels in pregnancies complicated by severe preeclampsia and in healthy normotensive pregnant controls.

Patients and methods

We carried out this study at the Department of Obstetrics and Perinatology Medical University Hospital in Lublin, Poland. The study design was approved by the institutional ethics committee.

The study was carried out on 43 pregnant patients with pregnancy complicated by severe preeclampsia (the PRE group). The control group consisted of 30 healthy normotensive pregnant patients with singleton uncomplicated pregnancies, without any renal, cardiac and vascular diseases and with normal laboratory tests (the C group).

The diagnosis of preeclampsia was established based on the presence of elevated systolic blood pressure of at least 140 mm Hg and diastolic blood pressure at least 90 mm Hg in women who were normotensive before 20 weeks of gestation, in association with proteinuria at least 300 mg/24h.

Severe preeclampsia was diagnosed on the basis of the following criteria: systolic blood pressure > 160 mm Hg, diastolic blood pressure >110 mm Hg on at least 2 occasions 6h apart with proteinuria > 5 g/in 24-h period. In addition patients with one or more of the following clinical manifestations: renal abnormalities (oliguria), hematologic abnormalities (thrombocytopenia and microangiopathic hemolysis) or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count and right-upper quadrant pain), or neurologic symptoms (headache, visual disturbances and seizures) were considered to have severe preeclampsia.

Preeclamptic patients were admitted to the Department of Obstetrics and Perinatology in the Medical University Hospital in Lublin because of the symptoms of the disease and without signs of labour. None of the pregnant patients with preeclampsia was affected by chronic hypertension or renal disorders and/or proteinuria before pregnancy and all were normotensive before 20th week of pregnancy. All arterial blood pressure measurements in the control group were normal and did not exceed 135/85 mmHg. Pregnant women with multiple pregnancies, diabetes, chorioamnionitis, and chronic renal diseases were also excluded from this study. All patients in the study were non-smokers.

Informed consent from the all studied patients was obtained for peripheral blood sampling.

Five milliliters of blood were collected by venipuncture from each preeclamptic patient and from each woman from the control group. They were centrifuged for 15 min at 500 × g.

The maternal serum soluble human leukocyte antigen-G levels were determined using a sandwich ELISA assay according to the manufacturer’s instructions (human a double monoclonal sandwich enzyme immunoassay, BioVendor Laboratorni Medicina a.s., Turnova, Czech Republic). A standard curve was performed to calculate the protein concentration of sHLA-G molecules. The minimum detectable sHLAG concentration was 1 Unit/ml, the intra-assay and inter-assay variations were < 5% and < 10%, respectively.

Data were presented as mean ± SD and were statistically analyzed with the computer program “Statistica 8”. The level of statistical significance was established as p < 0.05.

Results

Creatinine and urea levels were normal in all patients. Significant severe proteinuria (more than 5 g in 24h urine) was found only in patients from preeclamptic group.

There were no statistically significant differences in gravidity, parity, maternal age and height in patient profiles between studied groups of pregnant patients. Maternal weight and BMI were higher in preeclamptic patients than in normotensive healthy controls.

As expected preeclamptic patients presented significantly higher systolic and diastolic blood pressure compared with the control group. The mean systolic blood pressure values were $167.571 ± 15.762$ mm Hg in the group of women with pregnancy complicated by severe preeclampsia and $114.212 ± 11.266$ mm Hg in the con-
Serum soluble human leukocyte antigen-G in pregnancies complicated by severe preeclampsia

The mean diastolic blood pressure values were 109.524 ± 23.246 mmHg in the preeclamptic women and 72.788 ± 7.453 mmHg in the healthy controls. Lower gestational age was found in the group of preeclamptic women. The mean age of gestation was 34.296 ± 4.072 weeks in the PRE group and 38.525 ± 1.034 weeks in the healthy control subjects. Table 1 summarizes the characteristics of the pregnant women involved in the present study.

The levels of soluble HLA-G were elevated in the group of patients with pregnancy complicated by preeclampsia, but this difference was not statistically significant (p = 0.192182).

The mean values of sHLA-G levels were 28.385 ± 23.257 units/ml in the preeclamptic patients and 21.208 ± 22.410 units/ml in the healthy controls.

The results of this analysis are presented in Figure 1 and Table 1.

**Discussion**

The sHLA-G was detected not only in blood of pregnant women, but also in amniotic fluid and in the supernatant culture media from in vitro cultured embryos [18], but its role is still not clear. The production of soluble human leukocyte antigen G is associated with successful IVF therapy, suggesting that HLA-G production may be a marker for embryo quality [23]. Soluble form of HLA-G which has potentially wider range of activity than membrane-bound HLA-G performs exactly the same functions, not only locally but also systematically [18].

Yie et al. [19] found that low plasma HLA in early gestation correlates with later development of preeclampsia. It was suggested that insufficient trophoblast invasion was associated with the reduced expression of HLA-G. Therefore decreased levels of sHLA-G might be expected in patients with pregnancies complicated by severe preeclampsia.

However, in our study the levels of sHLA-G in maternal blood in pregnancies complicated by severe preeclampsia were higher than in healthy normotensive pregnant women.

But studies on circulating sHLA-G in preeclamptic pregnancies present various results and profound interindividual differences in sHLA-G1/G5 levels were observed in pregnant women affected by different pregnancy diseases and even in women with normal pregnancies [20, 26, 27].

According to Steinborn et al. [20] this phenomenon may be due to the fact that the different cell types may be the source of sHLA-G1 and/or sHLA-G5 molecules.

<table>
<thead>
<tr>
<th>Data</th>
<th>The control group (n = 30)</th>
<th>The PRE group (n = 43)</th>
<th>Statistical analysis control-PRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravidity</td>
<td>1.364 ± 0.653</td>
<td>1.634 ± 1.090</td>
<td>p = 0.213489</td>
</tr>
<tr>
<td>Parity</td>
<td>1.273 ± 0.452</td>
<td>1.463 ± 0.869</td>
<td>p = 0.257474</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>29.828 ± 4.025</td>
<td>30.391 ± 4.923</td>
<td>p = 0.607961</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>165.611 ± 6.634</td>
<td>164.960 ± 4.098</td>
<td>p = 0.693061</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>76.567 ± 13.858</td>
<td>86.556 ± 14.413</td>
<td>p = 0.030889*</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²)</td>
<td>27.884 ± 4.521</td>
<td>31.915 ± 5.148</td>
<td>p = 0.013199*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114.212 ± 11.266</td>
<td>167.571 ± 15.762</td>
<td>p &lt; 0.000001*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72.788 ± 7.453</td>
<td>109.524 ± 23.246</td>
<td>p &lt; 0.000001*</td>
</tr>
<tr>
<td>Age of gestation (weeks)</td>
<td>38.525 ± 1.034</td>
<td>34.296 ± 4.027</td>
<td>p &lt; 0.000001*</td>
</tr>
<tr>
<td>Umbilical sHLA-G levels (Units/ml)</td>
<td>21.208 ± 22.410</td>
<td>28.385 ± 23.257</td>
<td>p = 0.192182</td>
</tr>
</tbody>
</table>
In early pregnancy the main source of these molecules are probably fetal extravillous cytotrophoblastic cells which invade the maternal uterine arteries and therefore may come into close contact with the maternal circulation.

It is not known whether sHLA-G1/G5 molecules detected in maternal circulation are exclusively of placenta origin [6, 20].

But it was suggested that placenta may be a significant but not solely source of sHLA-G, because it was reported that the levels of sHLA-G in nonpregnant and pregnant women were very similar [6] and the concentration of sHLA-G does not increase during normal pregnancy [15, 22]. These findings suggest that the majority of the sHLA-G molecules detected in maternal circulation are produced by immunocompetent cells of mother.

According to Steinborn et al. [6] and Hviid [23] besides extravillous cytotrophoblast cells (G1), maternal or even fetal immune cells (monocytes, CD4+ T cells, CD8+ T cells and B cells) should be able to secrete the soluble forms of HLA-G as well. It is possible that increased numbers of emigrated fetal dendritic cells are responsible for the increased sHLA-G1/G5 levels [6, 20, 23].

Our results may suggest the important role of the sHLA-G in preeclampsia and support the hypothesis about disturbances in feto-maternal immune balance and may indicate the state of graft rejection in preeclamptic pregnancies.

Similar results were presented by Steinborn et al. [6, 20] and Le Rond et al. [24], who observed increased sHLA-G1/G5 levels in women with HELLP syndrome and partly in women with preeclampsia. Steinborn et al. [6] based on their findings concluded that HELLP syndrome may reflect the condition of acute graft rejection and may be associated with excessive alloreactive reaction against fetal antigens. These authors also suggest that higher levels of sHLA-G in pregnancy complicated by HELLP syndrome may be due to inflammatory reaction of the fetus which results in the increased release of fetally derived sHLA-G into maternal circulation [6].

But different results were presented by Hackmon et al. [25], who observed the decreased level of maternal HLA-G protein in third trimester preeclamptic pregnancies and suggested that this reduced concentrations of HLA-G may be related to early partial failure of maternal immune tolerance, which is manifested later as an inflammatory response.

Also different results were presented by Rizzo et al. [26], who found lower concentrations of sHLA-G1 in late pregnancies complicated by preeclampsia compared with uncomplicated pregnancies. They observed that higher concentrations of sHLA-G is not mandatory for a successful pregnancy, however pregnancy specific disorders, such as preeclampsia, spontaneous abortion, premature birth or IUGR are associated with low or undetectable levels of maternal sHLA-G, but in the same time higher levels of HLA-G5 form may exist in these pregnancy complications [26].

Hackmon et al. [10] observed that sHLA-G1 antigen expression is higher in amniotic fluid than in maternal-fetal compartments and significantly decreases toward term, that may stimulate a maternal immune response to the fetus and contribute to the initiation of parturition.

Probably HLA-G plays a significant role as a tissue protective molecule in inflammatory responses [27] and those higher levels of human leukocyte antigen may result from excessive inflammation. It was suggested that HLA-G can inhibit the transendothelial migration of NK cells [28] and can inhibit natural killer (NK)-cell-mediated cytolysis and Ag-specific CD8+ T cell-mediated cytolysis [29], shifts the cytokine balance towards Th2 dominance [30] and this way may limits the process of inflammation.

Lila et al. [31] observed that higher levels of sHLA-G in serum from patients after heart transplantation is associated with reduced number of acute graft rejection episodes and suggested that HLAG and its soluble form favour graft tolerance.

Because of these above observations higher sHLA-G levels in preeclamptic pregnancies observed in our study may result from higher inflammatory status in preeclamptic pregnancies, which induces higher production of sHLA-G. However this production may be insufficient to balance the excessive inflammatory process characteristic for preeclamptic pregnancies.

Our results may also suggest that the maintenance of the feto-maternal immune balance is disturbed in pregnancies complicated by severe preeclampsia.

The age of pregnancy was lower in preeclamptic patients than in normotensive healthy pregnant women from the control group, but it was found that maternal sHLA-G plasma levels do not change substantially in the course of pregnancy [6]. Steinborn et al. [6] presented that change in sHLA-G levels in pregnant women is not associated with age of gestation, but only with abnormal Doppler findings, which are used as an indicator for an insufficient trophoblast invasion during early pregnancy. Also Hunt et al. [32] did not observe any significant differences in values of sHLA-G levels obtained during the first, second, and third trimesters of pregnancy [32].
Conclusions
Our findings suggest that the increased levels of sHLA-G in the maternal serum in preeclamptic pregnancies may play a significant role in the aetiology and pathogenesis of preeclampsia. Elevated levels of sHLA-G in this pregnancy disorder may suggest that the maintenance of the feto-maternal immune balance is disturbed in preeclamptic pregnancies and may also be associated with the excessive inflammatory processes.

References
tinct fetal and maternal molecular pathways suggests a mechanism for the development of preeclampsia. J. Reprod. Immunol. 76: 54-60.


Marzena Laskowska
Chair and Department of Obstetrics and Perinatology
Medical University of Lublin
20-950 Lublin, ul. Jaczewskiego 8, Poland
e-mail: melaskowska@go2.pl